Methods for the Linkage-Group Determination of Insecticide-Resistance Factors in the Housefly

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planes for malathion in acetone and for that in acetone plus soy bean oil were $Y=-12.780+13.733x_1+3.091x_2$ and $Y=-13.721+13.733x_1+3.091x_2$ respectively. The results of tests for heterogeneity and for parallelism have shown no significant discrepancy. The mean probit difference was calculated to compare the mortality produced by two types of deposit. The result $A=0.941+0.149$ obtained means that the toxicity of malathion deposit for the house fly is decreased when the soy bean oil was added to acetone as solvent for impregnating the filter paper. Under the condition of this experiment deposit is a far more important factor than exposure time in determining the mortality. A doubling of deposit was here as effective as an increase in log time by $(b_1 \log 2)/b_2=1.327$ which corresponds to a multiplication of the time by 21.7.

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11. イエバエにおける殺虫剤抵抗性遺伝子の連鎖群決定法

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最近種々の衛生害虫や農業害虫において可視的なミュータントが報告され，その遺伝学的知見も次第に蓄積されてきたので，これらの昆虫においてもミュータントを用いて殺虫剤抵抗性を遺伝学的に研究していくことも可能となってきた．筆者らはここ数年間イエバエの各種殺虫剤に対する抵抗性について研究をおこなってきたので，それらの結果を報告するに先だって，抵抗性遺伝子の連鎖群決定に用いられた要因分析法について記載するとともに，他の害虫への応用に便ならしめた．

There are two major approaches for investigating the genetics of insecticide-resistance in insects: one is the toxicological examination of dosage-response data either at a single discriminating dose or at multiple scalar doses. The information to be derived from such a toxicological test in morphologically unmarked progeny of crossing experiments is rather indirect and inferential; Tsukamoto[13] has discussed the reliability of such log dosage-probit mortality (ld-p) relation in a previous paper. The other is the use of visible markers in crossing experiments and the examinations for segregants of these mutants. By such a method, the data available are more precise and the investigators can get rather direct information on the genetics of insecticide-resistance.

Recently several visible mutants have been reported in various insect pests of medical or agricultural importance, such as *Musca domestica* (by Milani[9], Sullivan and Hiroyoshi[12], Hiroyoshi[4], Tsukamoto *et al.*[14]); *Cochliomyia hominivorax* (by LaChance and Hopkins[7]); *Culex pipiens* (by Laven[8] and Kitzmiller[6]); *Aedes aegypti* (Craig and VandeHey[2] and VandeHey and Craig[16]); *Latheticus oryzae*, *Tribolium castaneum*, *T. confusum* (by Sokoloff[11]); *Blattella germanica* (by Cochran and Ross[1]); *etc.*; hence the genetic analysis of insecticide-resistance by means of visible mutants and statistical analysis now become possible to apply to these insect pests.

Most of the genetic analyses of insecticide-resistance by means of visible markers have been limited to *Drosophila* because of the extensive background of the formal genetics of species in this Genus. For determining the linkage groups of the genetic factor or factors investigated, certain statistical methods such as factorial analysis and subsequent analysis of variance have been employed by various geneticists (Crow[3]; King and Sømme[5]; Oshima and Hiroyoshi[10]; and Tsukamoto *et al.*[15]), but without any description of the actual procedure of factorial analysis which is less familiar to insect toxicologists.

The purpose of the present paper is, therefore, to describe the practical procedures which have been employed by the present author and his co-workers in genetic analysis of the housefly *Musca domestica* L. Although actual results

obtained with these analytical methods will be reported in detail in separate papers, preliminary data on BHC-resistance have been used as an example for the explanation of calculations. This is the second paper of the series of genetic studies on insecticide-resistance carried out in the laboratory at Osaka University.

Crossing Procedure

Unlike in *Drosophila*, no sex-linked visible mutant has yet been found out in the housefly while numbers of visible mutants have been located on all five autosomes. This evidence suggests that the X chromosome of the housefly is genetically almost or completely inert, and hence no sex-linked resistance factor may be expected. Furthermore, since preliminary experiments indicated a negligible maternal effect on the resistance level, no reciprocal crosses are attempted in subsequent experiments. The crossing procedures described here, therefore, are designed to determine the linkage group of autosomal resistance factors.

For determining the quantitative influence of particular chromosomes on the level of insecticide-resistance, it is necessary to use several multichromosomal mutant strains in which each autosome is marked with a visible mutant. Multichromosomally marked resistant strains are synthesized by crossing the unmarked resistant strain with susceptible mutant strains, and from the hybrid by making repeated backcrosses accompanied by selection both for visible mutant markers and for the resistance. In these special multichromosomal mutant strains, the mutant symbols are arranged in order of the linkage group and each linkage group is separated by the semicolons in parentheses as in *Drosophila*. For example, the notation R (*a*;*b*;*c*;*d*;*e*) stands for an insecticide-resistant strain in which the 2nd, 3rd, 4th, 5th and 6th chromosomes are marked with visible markers, *a*, *b*, *c*, *d* and *e*, respectively.

A schematic representation of typical crosssing procedures to detect both dominant and recessive resistance factor(s) is given in the next page. Figure 1 also illustrates the chromosomal constitutions in the F_1 male backcross systems to detect both dominant and recessive resistance factor(s).

1. Analysis of "dominant effect" of resistance factor

Males of the F_1 hybrid offspring of the S×R cross are backcrossed to females of the susceptible marker strain used. Adult flies of the resultant backcross progeny are then tested for their resistance levels by topical application of the insecticide or other appropriate methods. This procedure should detect any "dominant effect" of risistance factor(s) in the heterozygotes of the backcross offspring, viz. a comparison can

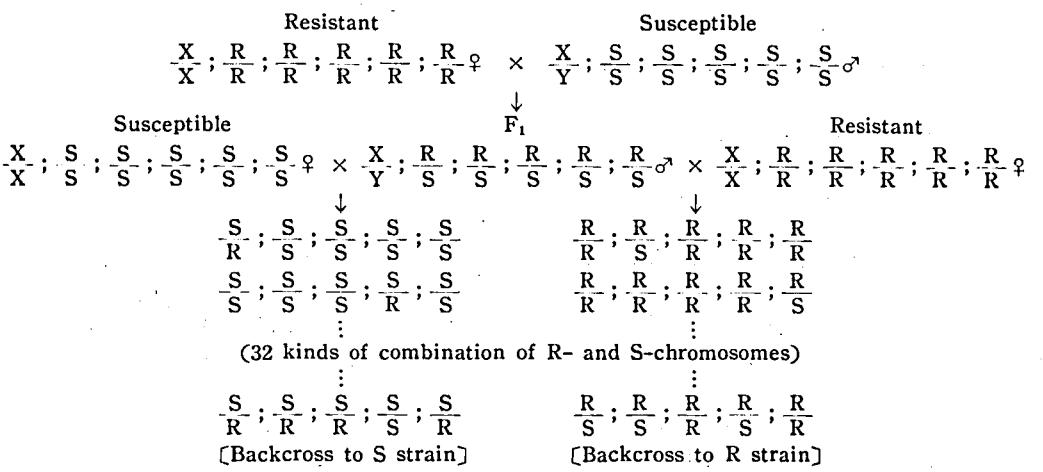

Figure 1. Schematic illustration of examples for backcrossing systems to detect both dominant and recessive resistant factors. R and S symbolize chromosomes derived from resistant and susceptible strains, respectively.

Cross 1. S(*a*;*b*;*c*;*d*;*e*)♀ × F_1{R(+;+;+;+;+)♀ × S(*a*;*b*;*c*;*d*;*e*)♂}♂
Cross 1′. S(*a*;*b*;*c*;*d*;*e*)♀ × F_1{S(*a*;*b*;*c*;*d*;*e*)♀ × R(+;+;+;+;+)♂}♂
Cross 2. S(*a*;*b*;*c*;*d*;*e*)♀ × F_1{R(*a*;*b*;*c*;*d*;*e*)♀ × S(+;+;+;+;+)♂}♂
Cross 2′. S(*a*;*b*;*c*;*d*;*e*)♀ × F_1{S(+;+;+;+;+)♀ × R(*a*;*b*;*c*;*d*;*e*)♂}♂
Cross 3. R(*a*;*b*;*c*;*d*;*e*)♀ × F_1{R(*a*;*b*;*c*;*d*;*e*)♀ × S(+;+;+;+;+)♂}♂
Cross 3′. R(*a*;*b*;*c*;*d*;*e*)♀ × F_1{S(+;+;+;+;+)♀ × R(*a*;*b*;*c*;*d*;*e*)♂}♂
Cross 4. R(*a*;*b*;*c*;*d*;*e*)♀ × F_1{R(+;+;+;+;+)♀ × S(*a*;*b*;*c*;*d*;*e*)♂}♂
Cross 4′. R(*a*;*b*;*c*;*d*;*e*)♀ × F_1{S(*a*;*b*;*c*;*d*;*e*)♀ × R(+;+;+;+;+)♂}♂

be made between the resistant heterozygote *R*/+ and the susceptible genotype +/+ among the siblings of either sex.

When the number of different marked chromosomes is *n*, segregation of 2^n different kinds of genotypes or phenotypes is expected in the backcross progeny with a theoretical ratio of 1:1:1:1:……:1. For example, if all 5 autosomes are marked with mutant characters, the following 32 kinds of combinations of different phenotypes may be expected in the backcross offspring:

1)	+;+;+;+;+	2)	*a*;+;+;+;+
3)	+;*b*;+;+;+	4)	*a*;*b*;+;+;+
5)	+;+;*c*;+;+	6)	*a*;+;*c*;+;+
7)	+;*b*;*c*;+;+	8)	*a*;*b*;*c*;+;+
9)	+;+;+;*d*;+	10)	*a*;+;+;*d*;+
11)	+;*b*;+;*d*;+	12)	*a*;*b*;+;*d*;+
13)	+;+;*c*;*d*;+	14)	*a*;+;*c*;*d*;+
15)	+;*b*;*c*;*d*;+	16)	*a*;*b*;*c*;*d*;+
17)	+;+;+;+;*e*	18)	*a*;+;+;+;*e*
19)	+;*b*;+;+;*e*	20)	*a*;*b*;+;+;*e*
21)	+;+;*c*;+;*e*	22)	*a*;+;*c*;+;*e*
23)	+;*b*;*c*;+;*e*	24)	*a*;*b*;*c*;+;*e*
25)	+;+;+;*d*;*e*	26)	*a*;+;+;*d*;*e*
27)	+;*b*;+;*d*;*e*	28)	*a*;*b*;+;*d*;*e*
29)	+;+;*c*;*d*;*e*	30)	*a*;+;*c*;*d*;*e*
31)	+;*b*;*c*;*d*;*e*	32)	*a*;*b*;*c*;*d*;*e*

Among these phenotypes, the symbol + indicates heterozygosity for the marker gene concerned (e. g. +/*b*) as well as heterozygosity with regard to R chromosome (+/*R*). Therefore, when a resistance factor is located on a particular chromosome, for example in the same linkage group as the marker *b*, the particular flies marked with the homozygous recessive mutant (*b*/*b*) should be susceptible to the insecticide concerned. Thus the linkage group to which the dominant resistance factor belongs may be directly detected by examining the visible marker characteristics of the survivors and the victims of a discriminating dose of the insecticide.

Although the case where *n*=3 or 4 is the most convenient for practical examinations of visible phenotypes of flies, all the possible combinations with five markers have been shown above for the schematic explanation. The arrangement of phenotypes listed above corresponds to that used in the factorial analysis which will be described later.

Other types of crossing system are also possible in cases where a multichromosomally marked resistant strain is available (Crosses 2 and 2′).

In these crosses, however, it is the phenotypes for the mutant in the particular linkage group concerned (e. g. *b*/*b*) that would be resistant (*R*/+) and the phenotypes for its wild-type allele (*b*/+) that would be susceptible (+/+) to the insecticide.

2. *Analysis of "recessive effect" of resistance factor*

When the resistance factor is recessive or incompletely dominant, a backcross of the F_1 males to a resistant marker strain should detect any "recessive effect" of the resistance factor(s), since it produces offspring containing resistant homozygotes for comparison with the heterozygotes. A schematic representation of such a backcross system is shown as Crosses 3 and 3′.

The following combinations in interstrain crosses are also possible, if necessary (Crosses 4 and 4′).

In these crossing systems, the mutant phenotypes in the progeny of crosses 3 and 3′ would be homozygous both for the resistance factor and for the mutant marker with which it is linked, while the wild-type phenotypes are heterozygous both for the resistance factor and for the marker for the chromosome in which it is located. Thus if a recessive resistance factor *r* is linked with marker gene *a*, then *a*/*a* genotypes in the

Table 1. Schematic arrangement of arc-sine transformed survival rates for statistical analysis.

Phenotype ($i=1, 2, \cdots, k$)	Dose or Replication ($j=1, 2, 3, \cdots\cdots, l$)					Sum $\Sigma\theta$	Mean $\bar{\theta}$
	1	2	3	j	l		
1	θ_{11}	θ_{12}	θ_{13}	θ_{1j}	θ_{1l}	$\theta_{1\cdot}$	$\bar{\theta}_{1\cdot}$
2	θ_{21}	θ_{22}	θ_{23}	θ_{2j}	θ_{2l}	$\theta_{2\cdot}$	$\bar{\theta}_{2\cdot}$
3	θ_{31}	θ_{32}	θ_{33}	θ_{3j}	θ_{3l}	$\theta_{3\cdot}$	$\bar{\theta}_{3\cdot}$
⋮	⋮	⋮	⋮		⋮	⋮	⋮
i	θ_{i1}	θ_{i2}	θ_{i3}	θ_{ij}	θ_{il}	$\theta_{i\cdot}$	$\bar{\theta}_{i\cdot}$
⋮	⋮	⋮	⋮		⋮	⋮	⋮
k	θ_{k1}	θ_{k2}	θ_{k3}	θ_{kj}	θ_{kl}	$\theta_{k\cdot}$	$\bar{\theta}_{k\cdot}$
Sum	$\theta_{\cdot 1}$	$\theta_{\cdot 2}$	$\theta_{\cdot 3}$	$\theta_{\cdot j}$	$\theta_{\cdot l}$	$\theta_{\cdot\cdot}$	$\bar{\theta}_{\cdot\cdot}$

progeny would survive a given discriminating dose of the insecticide. On the other hand, the heterozygotes for the marker gene are the homozygotes for the resistance factor in Crosses 4 and 4′.

Statistical Analyses of Data

The dominant or recessive effect of each "R chromosome" (the chromosome derived from the resistant strain) on the resistance level, or really of each resistance factor belonging to a particular linkage group, can be calculated by submitting the results of the crossing experiments to the usual factorial design developed by Yates[17].

Twenty-four hours after application of the discriminating dose of the insecticide, both the survivors and the dead flies are examined for their visible markers and are separated into their phenotype categories. The percentage survival rate recorded for each phenotype is then transformed into an arc-sine unit (θ) for statistical analysis. A schematic arrangement of the transformed data is shown in table 1, where k is the number of phenotypes and l is the number of replications at a single selective dose or the number of data at different doses of the insecticide. In order to submit the data to test of significance, it is necessary at least to be $l \geqq 2$.

The net effect of a given R chromosome (for example, A chromosome) on the resistance can be calculated subtracting the sum of mean survival rates ($\bar{\theta}$) of the a-type group from that of the A-type group. Combination of the mean survival rates is as follows:

Effect of the A chromosom
=(A−a)(B+b)(C+c)(D+d)(E+e)
= ABCDE−aBCDE+AbCDE−abCDE
+ABcDE −aBcDE +AbcDE −abcDE
+ABCdE −aBCdE +AbCdE −abCdE
+ABcdE −aBcdE +AbcdE −abcdE
+ABCDe −aBCDe +AbCDe −abCDe
+ABcDe −aBcDe +AbcDe −abcDe
+ABCde −aBCde +AbCde −abCde
+ABcde −aBcde +Abcde −abcde,

where each capital letter corresponds to each R chromosome in heterozygous(Crosses 1, 1′, 2 and2′) or homozygous (Crosses 3, 3′, 4 and 4′) condition, and each small letter corresponds to the S chromosome in homozygous (Crosses 1, 1′, 2, and 2′) or heterozygous (Crosses 3, 3′, 4, and 4′) condition. The relation between these symbols for the mean survival rate ($\bar{\theta}$) and visible phenotypes is summarized in table 2.

In a similar way, interactions between two or more resistance factors belonging to different linkage groups can also be calculated. For example, the interaction between resistance factors on the linkage groups B and C is as follows:

Interaction between the B and C chromosomes
=(A+a)(B−b)(C−c)(D+d)(E+e)
= ABCDE+aBCDE−AbCDE−abCDE
−ABcDE −aBcDE +AbcDE +abcDE
+ABCdE +aBCdE −AbCdE −abCdE
−ABcdE −aBcdE +AbcdE +abcdE
+ABCDe +aBCDe −AbCDe −abCDe
−ABcDe −aBcDe +AbcDe +abcDe
+ABCde +aBCde −AbCde −abCde
−ABcde −aBcde +Abcde +abcde.

Table 2. Schematic comparison between the symbols for the mean survival rate and phenotypes of the progeny.

Symbol	Mean survival rate	Phenotypes in backcross progeny	
		Crosses 1, 1′, 4 or 4′	Crosses 2, 2′, 3 or 3′
ABCDE	$\bar{\theta}_1$.	+;+;+;+;+	a;b;c;d;e
aBCDE	$\bar{\theta}_2$.	a;+;+;+;+	+;b;c;d;e
AbCDE	$\bar{\theta}_3$.	+;b;+;+;+	a;+;c;d;e
abCDE	$\bar{\theta}_4$.	a;b;+;+;+	+;+;c;d;e
⋮	⋮	⋮	⋮
abcdE	$\bar{\theta}_{16}$.	a;b;c;d;+	+;+;+;+;e
ABCDe	θ_{17}.	+;+;+;+;e	a;b;c;d;+
⋮	⋮	⋮	⋮
abcde	$\bar{\theta}_{32}$.	a;b;c;d;e	+;+;+;+;+

Table 3. Schematic representation of analysis of variance.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Total	$SS_T=\Sigma\theta_{ij}^2-\frac{\theta_{..}^2}{kl}$	$kl-1$	—	—
Phenotype	$SS_P=\frac{\Sigma\theta i.^2}{l}-\frac{\theta_{..}^2}{kl}$	$k-1$	$MS_P=\frac{SS_P}{k-1}$	$\frac{MS_P}{MS_E}$
Dose or Replication	$SS_D=\frac{\Sigma\theta.j^2}{k}-\frac{\theta_{..}^2}{kl}$	$l-1$	$MS_D=\frac{SS_D}{l-1}$	$\frac{MS_D}{MS_E}$
Error	$SS_E=SS_T-SS_P-SS_D$	$(k-1)(l-1)$	$MS_E=\frac{SS_E}{(k-1)(l-1)}$	—

Thus the effect of each R chromosome and the interaction between R chromosomes are actual quantitative function calculated in arc-sine units. Simultaneous calculations of these effects or interactions are performed practice with the convenient plus-minus method described by Yates[17]. Test for significance of these effects are based on analysis of variance, and when the effect of a particular R chromosome is statistically significant (at 5% level) or highly significant (at 1% level), it may be inferred that a resistance factor is associated with this chromosome.

The analysis of variance is performed by dividing the mean square for the phenotypes (MS_P) by the mean square for error (MS_E) to get the variance ratio (F-value) as shown schematically in table 3. When the calculated F for the phenotypes is greater than that expected at the 5% level, the sum of squares for the phenotypes (SS_P) are further subdivided into kl kinds of sum of squares for each chromosomal effect or interaction, where k ($=2^n$) is the number of phenotypes as already shown in table 1. The degree of freedom for each sum of squares is 1, and hence the sum of squares for each effect equals that of the mean square in this instance. The mean square for the effect of each R chromosome (or for interaction between combinations of R chromosomes) can be calculated from the value for effect already obtained in arc-sine units, as follows:

$$\frac{\text{Sum of squares}}{\text{Degree of freedom}}=\text{Mean square}=\frac{l(\text{Effect})^2}{k}$$

The variance ratio F for each effect or interaction can be obtained by dividing each mean square by that for the error (MS_E) in the usual manner. When the resistance to an insecticide is completely due to a single factor, only one chromosomal effect will be statistically significant in high degree. When two or more resistance factors act additively, two or more chromosomal effects will show significance. In cases where some factor acts synergistically with the major factor, an interaction between these chromosomal

Table 4. An example of genetic analysis for recessive BHC-resistance factors in the following backcross: HR(*bwb*;*ocra*;*ar*;*ac*)♀♀× F_1[HR(*bwb*;*ocra*;*ar*;*ac*)♀♀× Lab♂♂]♂♂

Phenotype (2 ; 3 ; 5 ; 6)	10-30 μg BHC/fly			50 μg BHC/fly			100-300 μg BHC/fly			Pooled $\Sigma\theta$	Mean $\bar{\theta}$
	No. used	Survival %	rate θ	No. used	Survival %	rate θ	No. used	Survival %	rate θ		
bwb ; *ocra* ; *ar* ; *ac*	29	51.72	45.98	15	46.67	43.09	26	38.46	38.32	127.39	42.46
+ ; *ocra* ; *ar* ; *ac*	30	10.00	18.44	35	11.43	19.76	18	5.56	13.63	51.83	17.28
bwb ; + ; *ar* ; *ac*	33	39.70	39.04	15	33.33	35.26	20	35.00	36.27	110.57	36.86
+ ; + ; *ar* ; *ac*	34	2.94	5.74	34	8.82	17.28	19	5.26	13.26	36.28	12.09
bwb ; *ocra* ; + ; *ac*	54	38.89	38.58	35	34.29	35.84	28	28.57	32.31	106.73	35.58
+ ; *ocra* ; + ; *ac*	44	6.82	15.14	31	3.19	10.27	18	0.00	0.00	25.41	8.47
bwb ; + ; + ; *ac*	51	21.57	27.67	48	27.08	31.36	25	24.00	29.33	88.36	29.45
+ ; + ; + ; *ac*	52	5.77	13.90	44	6.82	15.14	23	8.70	17.16	46.20	15.40
bwb ; *ocra* ; *ar* ; +	26	38.46	38.29	18	44.44	41.80	23	56.52	48.74	128.83	42.94
+ ; *ocra* ; *ar* ; +	26	26.92	31.25	34	14.71	22.56	13	15.38	23.09	76.90	25.63
bwb ; + ; *ar* ; +	28	28.57	32.31	31	25.81	30.54	18	27.78	31.81	94.66	31.56
+ ; + ; *ar* ; +	46	65.22	53.86	44	6.82	15.14	11	0.00	0.00	69.00	23.00
bwb ; *ocra* ; + ; +	43	51.16	45.67	52	44.23	41.69	12	33.33	35.26	122.62	40.87
+ ; *ocra* ; + ; +	28	35.71	36.70	36	0.00	0.00	22	9.09	17.55	54.25	18.08
bwb ; + ; + ; +	51	19.61	26.29	62	16.11	23.67	10	20.00	26.56	76.52	25.51
+ ; + ; + ; +	49	2.04	8.21	67	1.64	7.35	15	0.00	0.00	15.56	5.19
Total	624		477.07	601		390.75	301		363.29	1231.11	410.37

factors will be significant.

[Example]

For a convenience of explanation, an actual example of an analysis for the recessive effect of BHC-resistance in a Japanese resistant strain is employed below. Table 4 gives the 24-hours-mortality data after topical application with various doses of gamma-BHC in the following backcross progeny (this backcross corresponds to Cross 3 in the previous section):

HR(*bwb*;*ocra*;*ar*;*ac*)♀♀×

F_1[HR(*bwb*;*ocra*;*ar*;*ac*)♀♀×Lab♂♂]♂♂

where the resistant strain used is multichromosomally marked with the visible mutants, *bwb* (brown-body color, the 2nd chromosome), *ocra* (ocra eyes, the 3rd chromosome), *ar* (aristapedia, the 5th chromosome), and *ac* (ali curve, the 6th chromosome), respectively. Therefore, the analysis is effective to recessive resistance factors on all the autosomes but the 4th chromosome. From this crossing system, segregation of 16 kinds of phenotypes is expected ($k=2^4=16$), and the table contains data from three dose ranges ($l=3$).

The recessive effects of R chromosomes or chromosomal interactions have been calculated from the mean survival rate shown in the last column of table 4. The Yates' calculating procedure is given in table 5.

The analysis of variance has been done by calculating sum of squares as follows:

Total:

$$SS_T=45.98^2+18.44^2+39.04^2+\cdots\cdots+26.56^2-\frac{1231.11^2}{48}=9374.48$$

Phenotypes:

$$SS_P=\frac{127.39^2+51.83^2+\cdots\cdots+15.56^2}{3}-\frac{1231.11^2}{48}=6672.29$$

Doses:

$$SS_D=\frac{477.07^2+390.75^2+363.29^2}{16}-\frac{1231.11^2}{48}=440.65$$

Error:

$$SS_E=9374.48-6672.29-440.65=2261.54$$

Then the mean square for each effect or interaction has been calculated. For example, the mean square for the 2nd chromosomal effect is

$$\frac{3\times160.09^2}{16}=4805.40.$$ (See table 5)

Table 5. Calculation of chromosomal effects and interactions on the resistance.

Mean ($\bar{ii}$)	1	2	3	4 (Effect)	Chromosome
42.46		108.69	197.59	410.37	(Total)
17.28	→59.74 (+)	88.90	212.78	→160.09	2
36.86	48.95 (−)	123.13	→91.11	52.25 (+)	3
12.09	44.05	89.65	68.98	24.69 (−)	2×3
35.58	44.85	→49.95	9.99	53.27	5
8.47	68.57	41.16 (+)	42.26 (−)	−8.45	2×5
29.45	54.56	25.87	13.47	−2.65	3×5
15.40	58.95	43.11	11.22	−6.37	2×3×5
42.94	30.70	10.79	19.79	−15.19	6
25.63	→25.18 (+)	−0.80 (−)	33.48	→ 22.13	2×6
31.56	24.77 (−)	14.01	→ 8.79	−32.27	3×6
23.00	27.11	28.25	−17.24	2.25	2×3×6
40.87	14.05	→ 0.41	11.59	−13.69	5×6
18.03	17.31	13.06	−14.24	26.03	2×5×6
25.51	8.56	8.75	−12.65	25.83	3×5×6
5.19	22.79	2.47	6.28	−18.93	2×3×5×6
	20.32				

Table 6. Analysis of variance for the data given in tables 4 and 5.

S. V.	S. S.	D. F.	M. S.	F
Total	SS_T=9374.48	47	—	—
Phenotypes	SS_P=6672.29	15	444.82	5.90**
2	4805.40	1	4805.40	63.75**
3	511.89	1	511.89	6.79*
2×3	114.30	1	114.30	1.52
5	532.07	1	532.07	7.06*
2×5	13.39	1	13.39	0.18
3×5	1.32	1	1.32	0.02
2×3×5	7.61	1	7.61	0.10
6	43.26	1	43.26	0.57
2×6	91.83	1	91.83	1.22
3×6	195.25	1	195.25	2.59
2×3×6	0.95	1	0.95	0.01
5×6	35.14	1	35.14	0.47
2×5×6	127.04	1	127.04	1.69
3×5×6	120.78	1	120.78	1.60
2×3×5×6	67.19	1	67.19	0.89
Doses	SS_D=440.65	2	220.32	2.92
Error	SS_E=2261.54	30	75.38	—

* Significant at 5% level
** Significant at 1% level

The analysis of variance summarized in table 6 indicates that the 2nd chromosomal effect on the resistance is the most significant one at least among the chromosomes analyzed although the 3rd and the 5th chromosomal effects are also significant at 5% level. In other words, BHC-resistance is multifactorial and at least three recessive factors are responsible for the resistance in this

highly resistant strain. However, it is not a principal purpose to report or to discuss on the mode of inheritance of BHC-resistance in this section, but these unpublished data have been employed merely as an example for explaining the calculating procedures in the factorial analysis. Detailed results of the genetic analysis of BHC-resistance in the housefly will be described in a more complete form elsewhere.

Consideration

In the present paper, the symbols *R* and *r* have generally been used for dominant and recessive *resistance* factors respectively, and the symbol+ for either recessive or dominant *susceptible* alleles. Some investigators used feedlessly the symbol *r* for the *susceptible* allele of the dominant *resistant* factor *R*. Such a symbolization is, however, unsuitable to express a recessive *resistant* factor. In addition to these symbols, R and S are also used as general terms for resistant and susceptible strains or for the chromosome derived from the resistant strain (R chromosome) and from the susceptible strain (S chromosome), regardless of their dominance.

Since no crossing-over is observed in males of the housefly like *Drosophila*, determination of the linkage group of resistance factor(s) is based on backcrossing the F_1 male, so that each chromosome behaves as a unit in the crossing systems described above. Therefore, these factorial analyses are highly effective not only to determine the linkage group for the resistance factor qualitatively, but also to compare the relative effectiveness of each R chromosome or chromosomal interaction quantitatively. In some insects such as mosquitoes, however, the F_1 male backcross is not fully effective to detect the linkage group because the crossing-over occurs in both sexes of these insects. Thus genes located on different arms of a chromosome sometimes segregate independently of each other.

Together with the careful examination of dosage-mortality line described in the previous paper[13], these genetical and statistical analyses described in the present paper may bring more reliable informations on the genetics of insecticide-resistance.

Methods for the determination of locus of the resistance factor on the chromosome will be described in a separate paper.

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Summary

Methods and procedures of crossing experiments for determining the linkage group of both dominant and recessive effects of insecticide-resistance factor or factors by using visible multichromosomal mutant marker strains have schematically been described. Statistical treatment of the results obtained is then performed by the factorial analysis, to reveal those chromosomes which had a significant influence on the resistance level.

References Cited

1) Cochran, D. G. and Ross, M. H.: *Bull. Wld. Hlth. Org.*, **27**: 257~261 (1962).
2) Craig, G. B., Jr., and VandeHey, R. C.: *Ann. Ent. Soc. Amer.*, **55**: 47~58 (1962).
3) Crow, J. F.: *Ann. Rev. Ent.*, **2**: 227~246 (1957).
4) Hiroyoshi, T.: *J. Econ. Ent.*, **53**: 985~990 (1960).
5) King, J. C. and Sømme, L.: *Genetics*, **43**: 577~593 (1958).
6) Kitzmiller, J. B.: *Exp. Parasitol.*, **7**: 439~462 (1958).
7) LaChance, L. E. and Hopkins, D. E.: *J. Econ. Ent.*, **55**: 733~737 (1962).
8) Laven, H.: *Proc. Roy. Ent. Soc. Lond.*, **31**: 17~19 (1956).
9) Milani, R.: *Atti Intern. Congr. Genet., Caryologia Suppl.*: 791~796 (1954).
10) Oshima, C. and Hiroyoshi, T.: *Botyu-Kagaku*, **21**: 65~70 (1956).
11) Sokoloff, A.: *Insect Toxicol. Inform. Serv.*, **4**: 63~64 (1961).
12) Sullivan, R. L. and Hiroyoshi, T.: *J. Econ.*

Ent., 53: 213～215 (1960).
13) Tsukamoto, M.: *Botyu-Kagaku*, 28: 91～98 (1963).
14) Tsukamoto, M., Baba, Y. and Hiraga, S.: *Japan. J. Genet.*, 36: 168～174 (1961).
15) Tsukamoto, M., Ogaki, M. and Kikkawa, H., *Proc. Intern. Genet. Symp.*, 1956, *Suppl. Cytologia*: 442～444 (1957).
16) VandeHey, R. C. and Craig, G. B. *Jr.*: *Ann. Ent. Soc. Amer.*, 55: 58～69 (1962).
17) Yates, F.: *The design and analysis of factorial experiments.* Harpenden. (1937).

Cross Resistance in the "*Takatsuki*" Strain of the House fly, *Musca domestica vicina* selected with DDT. Studies on Insect Resistance to Insecticides. I. Hajime Ikemoto (Ihara Agricultural Chemicals Institute, Shimidzu.: Present Adress, Dept. of Agricultural Chemistry, Faculty of Agriculture, Nagoya University, Anjo) Received July 31, 1964. *Botyu-Kagaku*, 29, 59, 1964. (with English summary, 60.)

12. DDT で淘汰された高槻系イエバエにみられる交叉抵抗性

昆虫の殺虫剤抵抗性に関する研究 (第1報)* 池本 始** (イハラ農薬研究所) 39. 7. 31 受理

DDT で淘汰された高槻系イエバエの交叉抵抗性は欧米産イエバエのそれと違わなかった.

著者はさきに高槻系イエバエの2個体群を用いて DDT で淘汰をおこない, 抵抗性の発達はいちじるしく急速であることをあきらかにした[1),2)]. かようにして育成された抵抗性イエバエについて他の有機殺虫剤に対する交叉抵抗性を調査したのでつぎに報告する.

材料および実験方法

いずれの個体群も抵抗性の発達が平衡状態にたってから7・8代目のものを使用した. 抵抗性の発達が平衡にたってから更に10数代にわたって淘汰が試みられたが, 抵抗性はそれ以上に発達しなかった.

使用した殺虫剤の大部分は technical grade のものを再結 (蒸留) 精製し, 若干のものは合成したものである. すなわち, DDT (108～108.5), DDD 2,2-bis (p-chlorophenyl) 1,1 dichloroethane[3)] (109～109.5), DTDT 2,2-bis (p-tolylphenyl) 1,1,1 trichloroethane[4)] (86～87), DFDT 2,2-bis (p-fluorophenyl) 1,1,1 trichloroethane[5)] (44.5～45.0), DBrDT 2,2-bis (p-bromophenyl) 1,1,1 trichloroethane[3)] (141～142), Methoxychlor (88～89), DMC di (p-chlorophenyl) methylcarbinol[6)] (68～69), Lindane (112.5～113.5), Aldrin (104～105), Dieldrin (176～177), Isodrin (240～242), Methylparathion (36～36.5), Malathion (156/7mm), Dimethoate (51～52), Dipterex (79～81), DDVP (120/14mm), EPN (37), AC 5727 m-isopropylphenyl N-methylcarbamate (73～74), Sevin (141～142), a-dl-trans-allethrin (50～50.5) である. カッコ内は各殺虫剤の融点 (または沸点) °C をしめし, 各殺虫剤の肩につけた数字はそれぞれの文献によって合成したことをしめす. なお, 各殺虫剤の LD_{50} はさきに報告した方法[1)]によってもとめた.

実験結果および考察

第1表にしめすように塩素系殺虫剤とくに近縁の化合物にいちじるしい交叉抵抗性をしめす. DBrDT にたいしては DDT よりよわい感受性をしめすが, DFDT, DTDT および Methoxychlor にたいしては DDT よりも, つよい感受性をしめす. DDT 抵抗性の高槻系イエバエも抵抗性の原因はおもに DDT 脱塩酸酵素のたかい活性にもとづくと考えられるが, DFDT, DTDT および Methoxychlor が DDT よりもつよい殺虫力をしめすのは, これらの殺虫剤は DDT よりも DDT-脱塩酸酵素の働きをうけにくいためとおもわれる. 有機燐系殺虫剤およびピレトリン系殺虫剤に対する感受性の程度はことならない. なお, カーバメイト系殺虫剤 AC 5727 に対しても感受性の程度はあまりかわらない.

欧米に分布しているイエバエ *Musca domestica domestica* でもすでに DDT 抵抗性系統は塩素系殺虫剤とくに近縁の化合物につよい交叉抵抗性をしめすが, DFDT, DTDT, Methoxychlor などは DDT よりもつよい殺虫力をしめすこと[7)], 有機燐系殺虫剤およびピレトリン系殺虫剤にたいしては交叉抵抗性をしめさないこと[7)8)], Sevin につよい交叉抵抗性をし

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